Chronic Recording of Regenerating VIIIth Nerve Axons with a Sieve Electrode

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Experiment Team

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RATIONALE

One of the major goals of the Decade of the Brain (the 1990's) was the implementation of chronic interfaces to the nervous system. Such interfaces could serve as conduits for signal transmission past injured nerves or be used to control prostheses. One approach to this interface has been the sieve electrode. In this design, a small piece of silicon, with multiple small holes placed through it, is inserted into a nerve. Nerve fibers grow through the holes of the sieve and make contact with an electrode there. In this way, nerve traffic in the nerve fibers can be measured directly and signals can be introduced into the nerve. More than a quarter century has elapsed since the concept of the sieve electrode as a neural interface was first introduced, yet numerous technical difficulties have prevented its routine use. Hardware complications for a silicon-based wafer electrode have included difficulties in the consistent micromachining of quality electrodes, interfacing the microprobes with manageable recording leads, and developing transdermal (through the skin) connectors for continuous data acquisition. On the biological side, channeling of nerve fibers through the sieve pores has proven to be problematic even in nerves that exhibit vigorous regeneration.

The present study reports consistent success and repeated sampling of multiple, single-regenerating axons via a transdermal connector in awake, unrestrained fish. Long-term, chronic recording has been achieved by implanting a sieve electrode in the path of regenerating vestibular nerve fibers from the otolithic organs of a fish, *Opsanus tau* (oyster toadfish). This approach to nerve recording is ideal for spaceflight use (see science report by Boyle et al. in this publication) and also holds excellent promise for several clinical applications.

TECHNIQUE DESCRIPTION

The University of Michigan Center for Neural Communication Technology fabricated sieve electrodes possessing nine recording sites within a porous matrix using micromachining techniques (Figures 1A, B, C). The electrode consists of a recording head with a 20-μm-thick silicon support rim surrounding a four-μm-thick internal diaphragm. The diaphragm is interspersed with iridium-coated active sites (5–20 μm diameter) and additional noncoated pores (3–10 μm diameter). The active sites are integrated via silicon leads into a seven-mm silicon ribbon cable that terminates in a rectangular bonding pad that was ultrasonically bonded to a flexible circuit board, and connected via a transdermal lead to a nine-pin connector (PI Medical, Joure, The Netherlands). The impedance of each active site (0.2 to 1.5 MΩ) was determined before implantation, and regularly tested during recording sessions.

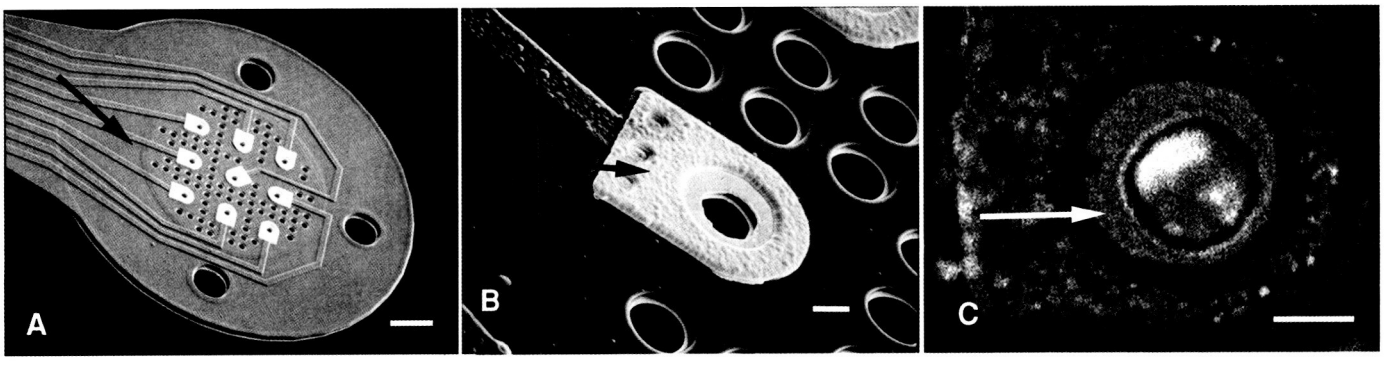
Initial implants were conducted using unmodified electrodes. Later implants incorporated several postproduction additions. The first was to electrostatically coat the electrode head with a porous thin film of ProNectin-L (SLPL) (Protein Polymer Technologies, Inc., San Diego, CA). A semipermeable nerve guide tube (NGT) (450-μm diameter, 400-μm total length) fabricated from poly- (acrylonitrile-) vinyl chloride was then attached to the head and secured with Loctite 3341 medical adhesive. Finally, the entubulated, coated probes were dipped into a neural adhesive [0.4% solution of protamine sulfate (Sigma) and poly-d, l-lysine (Sigma)] just prior to implantation.

Toadfish were obtained from the Marine Biological Laboratory in Woods Hole, MA, and maintained at 15°C in flow-through aquariums. All fish care and experimental procedures conformed to American Physiological Society and institutional guidelines.

Fish were anesthetized in 0.001% MS-222 (Sigma) and injected with 0.1 ml of 2% pancuronium bromide (Sigma). The vestibular (VIIIth) nerve was transected with iris scissors at one of two locations: (1) the anterior ramus of the VIIIth nerve, which included portions of semicircular canal and utricular nerves; or (2) the anterior portion of the saccular nerve. The sieve electrode was lowered into the transection site with a micromanipulator. The transected ends of the nerve were approximated to both sides of the electrode, or teased into NGTs. The bonding pad of the sieve electrode was mounted with cyanoacrylate gel to the cranium. The remainder of the cranium was sealed in a similar fashion, and the incision was sutured.

After surgery, the fish were tested biweekly for neural activity. Unrestrained and unanesthetized fish were placed in a small aquarium. The nine active sites of the sieve electrode were recorded differentially, and neural activity was recorded via a Cambridge Electronic Design 1401 computer interface.

For morphological examination, the toadfish were deeply anesthetized in 0.01% MS-222, and the tissues were preserved using perfusion fixation. The implant was examined to determine whether the electrode head was encapsulated with neural tissue. If so, the electrode and surrounding tissue were dissected from the brain and placed overnight in 20% sucrose



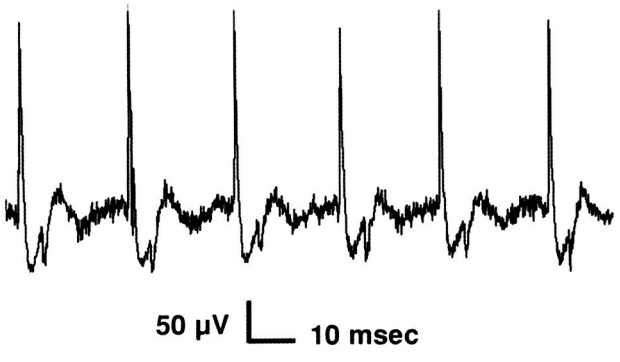


Figure 1. A. Scanning electron micrograph of the sieve microelectrode showing the nine active sites and the silicon leads (black arrows). The iridium-lined active sites were eight μm in diameter, and the unlined support pores were five to eight μm in diameter. B. Scanning electron micrograph of the eight-μm iridium-lined active site. C. Confocal micrograph of regenerating axons growing through an active site of an entubulated, SLPL probe (white arrow), 60 days following implantation. To help with visualization, the neural tissue has been reacted with a secondary tetramethylrhodamine antibody to neuron-specific enolase. D. Neural activity from the active site in Figure 1C 45 days following transection. Scale bars (μm): A=100; B=8; C= 5μV; D=5μV, 10 msec.

solution in phosphate buffer. The tissue/electrode block was then placed in an embedding medium and sectioned with a cryostat in 40-μm increments. The block was examined after each section to ascertain the presence of tissue contiguous with the electrode. If a gap in the tissue was detected before reaching within 200 μm of the electrode face, the implant was considered unsuccessful. If sectioning revealed a continuous line of tissue, the tissue/electrode block was sectioned to within 100-200 μm of the electrode face. The block was then reversed, and the process was repeated. The block was viewed with a Zeiss 510 laser scanning confocal microscope. Images were imported into Abode Photoshop and printed with a Kodak XLS printer.

Morphological examination demonstrated that 28 to 35 days was the minimum time needed for the axons to regenerate into or through the implant site, and that by the end of this period, the electrode head was routinely encased in neural tissue. The addition of SLPL increased the percentage of fibers that regenerated into the tubes, and established contact with the electrode face. Further success was attained with the addition of the nerve glue to the coated, entubulated electrodes.

A confocal micrograph taken from the distal face of an entubulated SLPL probe indicated that five of the nine active sites and approximately 50% of the nonlined pores are filled with neural material. Figure 1D shows a close-up of a 20-μm active site illustrating an axon traversing the pore (arrow). The neural recordings in Figure 1 were obtained from this site.

To date, seven implanted electrodes have yielded electrophysiological data. Three uncoated electrodes yielded spontaneous signals from single active sites following implantation in the anterior ramus of the VIIIth nerve. Four SLPL electrodes with NGTs, one in the saccular nerve and three in the utricular portion of the anterior ramus, yielded both spontaneous- and evoked-action potentials in response to mechanical stimulation. The earliest detection of neural activity occurred 29 days following nerve transection, with signals persisting for up to 42 days in individual fish.

Results demonstrate that axons will regenerate through a sieve electrode, and that chronic recordings are possible from these electrodes. Histological examination revealed that axons would grow on or through surfaces coated with extracellular matrix molecules such as the genetically engineered polymer SLPL. The polymer contains both GAGAGS crystalline silk-like sequences as well as several repeats of one of the identified laminin binding domains (IKVAV), making it a "super-sticky" laminin analogue. The filaments of polymer provided high surface area for cell attachment, and maintained the ability to transfer electrical signals between the nerves and the device. The NGTs channeled the nerves through the sieve, and the neural glue provided greater adhesion of the cut axons to the inside of the tube, greatly increasing the number of axons regenerating through the coated electrodes.

The present study was able to detect neural activity within 29 days of transection with a median delay of 42 days post-transection. This was significantly faster than the 49- to 175-day intervals reported in previous studies, emphasizing the advantage of transdermal leads for repeated sampling. Although morphological examination determined that up to seven active sites contained neural tissue, the maximum number of active sites that yielded neural activity was three in any one fish. It is assumed that electrical activity can only be recorded from sites in proximity to nodes of Ranvier. As the axons regenerate through the pores, the nodes of Ranvier would be distributed at different distances along the axons. Although the internodal distance is foreshortened in regenerating nerves, thereby increasing the chances of a node being near an active site, the minimum distance a node can be situated from an active site and produce viable recordings remains to be determined. The variability in internodal distance may explain why the number of recording sites remained less than the number of active sites containing neural material.

Experiments have demonstrated that regenerating axons will grow through the sieve electrode, and that neural recording is possible from unrestrained animals.. The eventual integration of the sieve electrode with a telemetry device will allow chronic recording from freely moving animals, and determine how the nervous system functions in a natural environment. This information should greatly enhance our knowledge of the neural mechanisms of behavior.

APPLICATION

The device holds excellent promise for several clinical applications. The ability of mammalian peripheral nerves to partially regenerate following injury suggests that the device could serve as a conduit for signal transmission past injured nerves. In systems that have limited natural regeneration, such as spinal cord, neurotrophins could be incorporated with the SLPL molecule to stimulate sufficient growth to have nerve sprouts enter the electrode and establish a neural interface for prosthesis control.

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